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Molecular Basis of Erythroenzymopathies Associated With Hereditary Hemolytic Anemia: Tabulation of Mutant Enzymes

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Molecular abnormalities of erythroenzymopathies associated with hereditary hemolytic anemia have been determined by means of molecular biology. Pyruvate kinase (PK) deficiency is the most common and well-characterized enzyme deficiency in the glycolytic pathway, and it causes hereditary hemolytic anemia. To date, 47 gene mutations have been identified. We identified one base deletion, one splicing mutation, and six distinct missense mutations in 12 unrelated families with a homozygous PK deficiency. Mutations located near the substrate or fructose-1,6-diphosphate binding site may change the conformation of the active site, resulting in a drastic loss of activity and severe clinical symptoms. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common metabolic disorder, and it is associated with chronic hemolytic anemia and/or drug- or infection-induced acute hemolytic attack. An estimated 400 million people are affected worldwide. The mutations responsible for about 78 variants have been determined. Some have polymorphic frequencies in different populations. Most variants are produced by one or two nucleotide substitutions. Molecular studies have disclosed that most of the class 1 G6PD variants associated with chronic hemolysis have the mutations surrounding either the substrate or the NADP binding site. Among rare enzymopathies, missense mutations have been determined in deficiencies of glucosephosphate isomerase, phosphofructokinase (PFK), aldolase, triosephosphate isomerase (TPI), phosphoglycerate kinase, and adenylate kinase. Compound heterozygosity with missense mutation and base deletion has been determined in deficiencies of hexokinase and diphosphoglyceromutase. Compound heterozygosity with missense and nonsense mutations has been identified in TPI deficiency. One base deletion resulting in a frameshift and the premature termination of translation and splice junction mutations resulting in abnormally spliced PFK-M mRNA have been identified in homozygous PFK deficiency. An exception is hemolytic anemia due to increased adenosine deaminase activity. The basic abnormality appears to result from the overproduction of a structurally normal enzyme. © 1996 Wiley-Liss, Inc.

Key words: PK deficiency, hereditary hemolytic anemia, mutant enzymes

INTRODUCTION

Since the discovery of glucose-6-phosphate dehydrogenase (G6PD) and pyruvate kinase (PK) deficiencies, erythroenzymopathies associated with hereditary hemolytic anemia have been extensively investigated [1,2]. Normal genomic DNA and cDNA for most of the enzymes causing hereditary hemolytic anemia have been isolated [3,4], rapidly advancing understanding of the molecular basis of erythroenzymopathies. Molecular abnormalities determined are listed in Table I. They are mostly missense mutations. Nonsense mutation, gene deletion, gene insertion, and splicing mutation have also been found in several variant enzymes.

This review summarizes the recent progress in the molecular genetic analysis of erythroenzymopathies.

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TABLE I. Molecular Abnormalities of Erythroenzymopathies Associated With Hereditary Hemolytic Anemia

Enzyme	Molecular defects				
	Missense	Nonsense	Deletion	Insertion	Splicing
Hexokinase	1	0	1	0	0
Glucosephosphate isomerase	12	0	0	0	0
Phosphofructokinase	8	0	1 (fs-pt;1)	0	4
Aldolase	1	0	0	0	0
Triosephosphate isomerase	4	1	0	0	0
Diphosphoglyceromutase	1	0	1 (fs-pt;1)	0	0
Phosphoglycerate kinase	5	0	0	0	1
Pyruvate kinase	33	3	4 (fs-pt;2)	3 (fs-pt;1)	4
Glucose-6-phosphate dehydrogenase	71	1	5	0	1
Adenylate kinase	1	0	0	0	0
Total	137	5	12	3	10

fs-pt, frameshift → premature termination.

PYRUVATE KINASE DEFICIENCY

Pyruvate kinase (PK), a key enzyme in the glycolytic pathway, has four isozymes in mammals: M_1, M_2, L, and R. The R-type isozyme is exclusively expressed in red cells. Kinetic, electrophoretic, and immunological properties suggest that the L and R types differ from M_1 and M_2 types and that these two isozymes are probably under the control of different genes. We cloned human L- and R-type PK cDNAs and the structural gene for both isozymes, the L-PK gene [5,6]. A structural analysis revealed that L- and R-type PK are encoded in the single gene and that tissue-specific promoters generate mRNAs with different 5′-end sequences. The isozyme switches from the M_2 to R-type during erythroid differentiation. The switching achieved by activating the R-PK promoter activity and by involving erythroid-specific transcription factor(s) has been demonstrated [6]. R-type PK cDNA is 2,039 bp long and encodes 574 amino acids, 31 more than the L-type at the NH_2-terminal. The L-PK gene has 12 exons spanning about 11.5 kb and has been assigned to chromosome 1 (1q21).

Glycolysis is a major energy source for red cells. Therefore, a PK deficiency results in hemolysis. PK deficiency is the most common and well-characterized enzyme deficiency involving the glycolytic pathway. It is inherited in autosomal recessive manner [7]. Nearly 400 patients with PK deficiency have been described [2]. Anemia is moderate to severe, and some individuals with severe PK deficiency require exchange transfusion or splenectomy during the neonatal period, or death occurs during the early infantile period, as found among the Amish [8]. Most PK variants are compound heterozygotes of mutant PK genes, whereas some homozygotes caused mostly by consanguineous marriage have been reported. Residual erythrocyte PK activity is not usually associated with phenotypic severity, whereas enzymatic characteristics such as decreased substrate affinity, thermal instability, or impaired response to the allosteric activator, fructose-1,6-diphosphate (FDP) correspond well with the phenotype.

To date, 47 gene mutations have been identified [5,8–21] (Tables I, II), and a molecular approach appears to be helpful for diagnosing a PK deficiency, particularly among transfusion-dependent individuals or infants. We analyzed PK genes responsible for hereditary hemolytic anemia among Japanese, American and Chinese homozygous PK variants by cDNA or genomic DNA cloning. Among 12 homozygous PK-deficient families, 6 distinct missense mutations, a one-base deletion, and a splicing mutation have been identified. These mutations locate near the substrate binding site, or at exon 10, which is important for both the intersubunit contact and allosteric activity. It is considered that these variant enzymes change the conformation of the active site or the tetramer formation of PK subunits, resulting in a drastic loss of activity. We produced human PK with a single amino acid substitution (^{384}Thr to Met) in *Escherichia coli* system and showed that the variant PK had marked thermal instability in vitro [5]. Another group found point mutations in homozygous Turkish and Lebanese PK-deficient individuals, and one mutation in the Lebanese was identical with that identified in two Japanese families [10].

In PK Beppu, one of the most severe PK variants, the M_2-type PK persists in mature red cells and in the liver. We found that the variant was homozygous with a one base deletion (434 C del) of the L-PK gene, resulting in a frameshift and premature termination of translation [9]. The truncated R-PK subunit lacks about two-thirds of the C-terminal portion, and has no catalytic activity. The affected red cells may survive by means of compensatory M_2-PK expression.

A point mutation in the 5′-donor site of intron 7 of the human PK-L gene has been identified in nonidentical Nepalese twin girls with PK Kowloon who are transfusion

TABLE II. Molecular Abnormalities of Pyruvate Kinase Deficiency

Mutant enzyme	Heredity	DNA defect	Deduced defect	Defect of other allele	Reference
PK Beppu	Homo	434 C del	fs-pt	434 C del	9
PK Linz	Homo	487 C→T	163 Arg→Cys	487 C→T	10
PK Kowloon	Homo	IVS-7(+1)G→T	Splicing mutation	IVS-7(+1)G→T	11
PK Hong Kong	Homo	941 T→C	314 Ile→Thr	941 T→C	11
PK Tokyo Nagasaki, Beirut	Homo	1151 C→T	384 Thr→Met	1151C→T	5,10,12,13
PK Fukushima Maebashi, Sendai	Homo	1261 C→A	421 Gln→Lys	1261 C→A	12,14
PK Naniwa	Homo	1276 C→T	426 Arg→Trp	1276 C→T	9
PK Sapporo	Homo	1277 G→A	426 Arg→Gln	1277 G→A	15
PK	Homo	1378 G→A	460 Val→Met	1378 G→A	16
PK Hadano	Homo	1403 C→T	468 Ala→Val	1403 C→T	14
PK Amish, Shinshu	Homo	1436 G→A	479 Arg→His/splicing	1436 G→A	8,14
PK Gypsy	Homo	1437–1618 del	fs-pt	1437–1618 del	17
PK	Homo	1529 G→A	510 Arg→Gln	1529 G→A	18
PK	Hetero	IVS-2(−1)G→A	Splicing mutation	1493 G→A	18
PK	Hetero	238 T→C	80 Ser→Pro	1468 C→T	19
PK	Hetero	307 C del	Nonsense	1378 G→A	16
PK	Hetero	391–393 ATC del	131 Ile del	721 G→T	17
PK	Hetero	401 T→C	134 Val→Asp	1456 C→T	17
PK	Hetero	464 T→C	155 Leu→Pro	1529 G→A	17
PK	Hetero	664–666 GAC ins	222 Asp ins	1468 C→T	9
PK	Hetero	721 G→T	Nonsense	1529 G→A	17
PK	Hetero	808 C→T	Nonsense	1529 G→A	16
PK	Hetero	841 G→A	281 Asp→Asn	1698 C→A	9
PK	Hetero	859 T→G	287 Phe→Val	?	9
PK	Hetero	993 C→A	331 Asp→Glu	1529 G→A	17
PK	Hetero	994 G→A	332 Gly→Ser	1529 G→A	18
PK	Hetero	1006 G→T	336 Ala→Ser	?	18
PK	Hetero	1022 G→C	341 Gly→Ala	1456 C→T	17
PK	Hetero	1060–1062 AAG del	354 Lys del	1594 C→T	18
PK	Hetero	1075 C→T	359 Arg→Cys	?	9
PK	Hetero	1076 G→A	359 Arg→His	?	17
PK	Hetero	1081 A→G	361 Asn→Asp	?	18
PK	Hetero	1089 G ins	fs-pt	1529 G→A	16
PK Osaka	Hetero	1102 G→T	368 Val→Phe	Reduced mRNA	20
PK	Hetero	1174 G→A	392 Ala→Thr	?	18
PK	Hetero	1178 A→G	393 Asn→Ser	1456 C→T	16
PK	Hetero	1179 T→A	393 Asn→Ser	1456 C→T	16
PK	Hetero	1204–1206 AGC ins	402 Ser ins	1529 G→A	18
PK	Hetero	1269 G→A	Splice site mutation	?	9
PK	Hetero	1269 G→C	Splice site mutation	?	21
PK	Hetero	1373 G→A	458 Gly→Asp	1529 G→A	17
PK	Hetero	1456 C→T	486 Arg→Trp	1178 A→G	17
PK	Hetero	1468 C→T	490 Arg→Trp	238 T→C	9,19
PK	Hetero	1484 C→T	495 Ala→Val	1529 G→A	17
PK	Hetero	1493 G→A	498 Arg→His	1456 C→T	16,18
PK	Hetero	1594 C→T	532 Arg→Trp	1060–1062 AAG del	18
PK	Hetero	1698 C→A	566 Asn→Lys	841 G→A	9

PK, pyruvate kinase; Homo, homozygote; Hetero, heterozygote; del, deletion; fs-pt, frameshift→premature termination; ins, insertion.

dependent [11]. The +1 position of intron 7 was replaced from GT to TT, resulting in retention of intron 7 in the R-PK mRNA. Consequently, the translational product may lack one-third of the C-terminal portion of the R-PK, since premature termination would occur in the region encoded by intron 7 sequence.

Although the PK isozyme switches from M_2 to R-type during normal erythroid maturation, R-PK has rarely been observed in erythrocyte lysates from patients with a severe PK deficiency. We analyzed the PK genes of a member of a family with PK Osaka and identified a missense mutation near the active site in the PK alleles of both the proband and her father [20]. The R-PK mRNA content in the proband measured by means of PCR revealed that R-PK mRNA in her reticulocytes was decreased to 60% of that in reticulocyte-rich controls. This result suggested

that another mutation, probably inherited from her mother, is involved in the region that regulated transcriptional activity in erythroid cells.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

G6PD is the initial and rate-limiting enzyme of the pentose phosphate pathway. Human G6PD monomer consists of 515 amino acids, including the initial methionine residue. Only the tetrameric or dimeric forms composed of a single type subunit are catalytically active. In human erythrocytes, the dimers are the predominant form. The main function of G6PD, particularly in erythrocytes, is to produce the potent antioxidant NADPH, which is essential for maintaining glutathione in the reduced state. The gene for G6PD is located on the X chromosome.

A hereditary deficiency of G6PD is one of the most common genetic disorders, with more than 400 million people affected worldwide. G6PD deficiency is caused by the production of variant enzymes with abnormal properties. Each variant causes various degrees of enzyme deficiency, and they are associated with hemolytic anemia over a range of clinical severity, from chronic hemolytic anemia, to drug-induced acute hemolysis, to being completely asymptomatic. In 1967, a committee of the World Health Organization (WHO) proposed standard procedures to characterize variants using parameters such as enzyme activity, electrophoretic mobility, the K_m for glucose-6-phosphate (G6P) and NADP, the use of substrate analogues, heat stability, and pH optimum [22]. Thereafter, the properties of variants identified in different laboratories were compared. The relationship between the enzymological properties of G6PD variants and clinical severity is somewhat ambiguous. A low inhibition constant (K_i) for NADPH [23], increased K_m for G6P [24], or decreased heat stability [25] have been proposed as important causative factors of chronic hemolytic anemia. Our preliminary statistical analysis suggested a correlation between low K_i NADPH and chronic hemolysis; 10 variants out of 12 with apparently low K_i NADPH belonged to class 1.

The mutations responsible for 78 variants have been determined [26–71] (Tables I, III). Some of them have polymorphic frequencies in different populations. Most of the variant enzymes are produced by one or two missense mutations in the structural gene. G6PD Vancouver [41] is caused by three nucleotide substitutions. Although nucleotide deletions or nonsense mutations are common molecular abnormalities that may cause a variety of genetic disorders, they are rare in G6PD deficiency, except for G6PD Georgia [54], which is a heterozygote of nonsense mutation. In-frame nucleotide deletions have been found only in five variants: G6PD Sunderland [29], G6PD Urayasu [40], G6PD Tsukui [40], G6PD Stonybrook [54], and G6PD Nara [60], each of which causes a deletion of not more than eight amino acid residues. Abnormal splicing due to deletion in the 3′ intron 10 at the C-terminal position have been found [54]. The low frequency of amino acid deletion as a cause of G6PD deficiency implies that the severe tissue dysfunction usually associated with such a drastic structural aberration is presumably lethal unless the involved region is functionally insignificant.

Molecular analysis of G6PD variants combined with standard characterization has provided several interesting findings regarding the structure–function relationship of the enzyme [72]. For example, G6PD Tomah, G6PD Iowa, G6PD Beverly Hills, and G6PD Nashville are unique variants that lose virtually all activity during purification but can be reactivated by a high concentration of NADPH and magnesium. The amino acid substitutions of these variants are located in a region of nine amino acids, and the region is involved in the "structural" NADP binding site. G6PD Tomah, G6PD Beverly Hills, G6PD Nashville, and G6PD Riverside have an unusually high K_m for NADP. This suggests that this region also serves as a "catalytic" NADP binding site. G6PD Puerto Limon and G6PD Riverside also have amino acid substitutions near the NADP binding sites. The latter is slightly reactivated by a high concentration of NADP. The very high K_m G6P of G6PD Nashville and G6PD Riverside has led to the notion that there is some interaction between the NADP and G6P binding sites. It is notable that the mutations of most of class 1 variants associated with chronic hemolysis are clustered surrounding the putative "structural" NADP binding site. This indicates that the region is closely related to the important function of the enzyme.

A model of the tertiary and quaternary structure of human G6PD has been constructed based on the solved structure of G6PD from *Leuconostoc mesenteroides,* which has 33% sequence homology to the human enzyme. The substrate and coenzyme binding sites and the regions involved in the dimer interface have been identified [73]. This study may provide useful information to explain the genotype–phenotype relationship of G6PD deficiency.

OTHER ENZYME DEFICIENCIES WITH DEFINED MOLECULAR DEFECTS

Many enzyme deficiencies, including hexokinase, glucosephosphate isomerase, phosphofructokinase, aldolase, triosephosphate isomerase, diphosphoglyceromutase, phosphoglycerate kinase, and adenylate kinase, have been characterized at the molecular level, as listed in Tables I and IV [74–103].

Fifteen unrelated families with a hexokinase deficiency have been reported. Most show only hemolysis, but some manifested associated disorders such as multiple malformation, latent diabetes mellitus, and psychomotor retarda-

TABLE III. Molecular Abnormalities of Glucose-6-Phosphate Dehydrogenase Deficiency

Mutant enzyme	Heredity	DNA defect	Deduced defect	Reference
G6PD Gaohe	Hemi	95 A→G	32 His→Arg	26,27
G6PD Honiara	Hemi	99 A→G + 1360 C→T	33 Ile→Met + 454 Arg → Cys	28
G6PD Sunderland	Hemi	105–107 del	35 Ile del	29
G6PD Aures	Hemi	143 T→C	48 Ile→Thr	30
G6PD Metaponto	Hemi	172 G→A	58 Asp→Asn	31
G6PD A(−)	Hemi	202 G→A + 376 A→G	68 Val→Met + 126 Asn→Asp	32–35
G6PD Namouru	Hemi	208 T→C	70 Try→His	36
G6PD Murcia	Hemi	209 A→G	70 Try→Cys	37
G6PD Swansea	Hemi	224 T→C	75 Leu→Pro	38
G6PD Konan	Hemi	241 C→T	81 Arg→Cys	39
G6PD Lagosanto	Hemi	242 G→A	81 Arg→His	38
G6PD Urayasu	Hemi	281–283 del	95 Lys del	40
G6PD Vancouver	Hemi	317 C→G + 544 C→G + 592 C→T	106 Ser→Cys + 182 Arg→Trp + 198 Arg→Cys	41
G6PD São Borga	Hemi	337 G→A	113 Asp→Asn	42
G6PD A(+)	Hemi	376 A→G	126 Asn→Asp	43
G6PD Santamaria	Hemi	376 A→G + 542 A→T	126 Asn→Asp + 181 Asp→Val	35
G6PD A(−)	Hemi	376 A→G + 680 G→T	126 Asn→Asp + 227 Arg→Leu	32
G6PD A(−)	Hemi	376 A→G + 968 T→C	126 Asn→Asp + 323 Leu→Pro	32,38
G6PD Mt. Sinai	Hemi	376 A→G + 1159 C→T	126 Asn→Asp + 387 Arg→Cys	38
G6PD Vanua Lava	Hemi	383 T→C	128 Leu→Pro	36
G6PD Quing Yuan	Hemi	392 G→T	131 Gly→Val	26
G6PD Ilesha	Hemi	466 G→A	156 Glu→Lys	31
G6PD Mahidol	Hemi	487 G→A	163 Gly→Ser	44
G6PD Plymouth	Hemi	488 G→A	163 Gly→Asp	38
G6PD Taiwan-Hakka	Hemi	493 A→G	165 Asn→Asp	45
G6PD Naone	Hemi	497 G→A	166 Arg→His	36
G6PD Shinshu	Hemi	527 A→G	176 Asp→Gly	46
G6PD Tsukui	Hemi	561–563 del	188 Ser del	40
G6PD Mediterranean	Hemi	563 C→T	188 Ser→Phe	31,38,47,48
G6PD Coimbra	Hemi	592 C→T	198 Arg→Cys	30
G6PD Santiago	Hemi	593 G→C	198 Arg→Pro	49
G6PD Sibari	Hemi	634 A→G	212 Met→Val	50
G6PD Minnesota	Hemi	637 G→T	213 Val→Leu	51,52
G6PD Harilaou	Hemi	648 T→G	216 Phe→Leu	53
G6PD Mexico City	Hemi	680 G→A	227 Arg→Gln	49
G6PD Stonybrook	Hemi	724–729 del	242–243 del	54
G6PD Wayne	Hemi	769 C→G	257 Arg→Gly	52
G6PD Cleveland	Hemi	820 G→A	274 Glu→Lys	54,55
G6PD Wexham	Hemi	833 C→T	278 Ser→Phe	55
G6PD Chinese-1	Hemi	835 A→T	279 Thr→Ser	56
G6PD Seattle	Hemi	844 G→C	282 Asp→His	48,50,57
G6PD Montalbano	Hemi	854 G→A	285 Arg→His	50,58
G6PD Viangchan	Hemi	871 G→A	291 Val→Met	35,38,52
G6PD West Virginia	Hemi	910 G→T	303 Val→Phe	54
G6PD Kalyan	Hemi	949 G→C	317 Glu→Lys	59
G6PD Nara	Hemi	953–976 del	319–326 del	60
G6PD Chatham	Hemi	1003 G→A	335 Ala→Thr	31
G6PD Fushan	Hemi	1004 C→A	335 Ala→Asp	54
G6PD Mahidol-like	Hemi	1024 C→T	342 Leu→Phe	26
G6PD Partenope	Hemi	1052 G→T	351 Gly→Trp	61
G6PD Ierapetra	Hemi	1057 C→T	353 Pro→Ser	49
G6PD Loma Linda	Hemi	1089 C→A	363 Asn→Lys	51
G6PD Olomouc	Hemi	1141 T→C	381 Phe→Leu	54
G6PD Tomah	Hemi	1153 T→C	385 Cys→Arg	31
G6PD Iowa	Hemi	1156 A→G	386 Lys→Glu	62
G6PD Guadalajara	Hemi	1159 C→T	387 Arg→Cys	49
G6PD Beverly Hills	Hemi	1160 G→A	387 Arg→His	35,62
G6PD Praba	Hemi	1166 A→G	389 Glu→Gly	54
G6PD Nashville	Hemi	1178 G→A	393 Arg→His	51,63

continued

TABLE III. Molecular Abnormalities of Glucose-6-Phosphate Dehydrogenase Deficiency (Continued)

Mutant enzyme	Heredity	DNA defect	Deduced defect	Reference
G6PD Alhambra	Hemi	1180 G→C	394 Val→Leu	49
G6PD Puerto Limon	Hemi	1192 G→A	398 Glu→Lys	64
G6PD Clinic	Hemi	1215 G→A	405 Met→Ile	37
G6PD Riverside	Hemi	1228 G→T	410 Gly→Cys	62
G6PD Shinagawa	Hemi	1229 G→A	410 Gly→Asp	46,49
G6PD Tokyo	Hemi	1246 G→A	416 Glu→Lys	65
G6PD Georgia	Hetero	1284 C→A	Nonsense	54
G6PD Varnsdorf	Hemi	IVS-10(3′ end)AG del	Splicing mutation	54
G6PD Pawnee	Hemi	1316 G→C	439 Arg→Pro	49
G6PD Kobe	Hemi	1318 C→T	440 Leu→Phe	66
G6PD Santiago de Cuba	Hemi	1339 G→A	447 Gly→Arg	31
G6PD S. Antioco	Hemi	1342 A→G	448 Ser→Gly	61
G6PD Cassano	Hemi	1347 G→C	449 Gln→His	50
G6PD Maewo	Hemi	1360 C→T	454 Arg→Cys	28,50,56,67
G6PD Andalus	Hemi	1361 G→A	454 Arg→His	68
G6PD Cosenza	Hemi	1376 G→C	459 Arg→Pro	50
G6PD Canton	Hemi	1376 G→T	459 Arg→Leu	69,70
G6PD Kaiping	Hemi	1388 G→A	463 Arg→His	70
G6PD Campinas	Hemi	1463 G→T	488 Gly→Val	71

G6PD, glucose-6-phosphate dehydrogenase; Hemi, hemizygote; Hetero, heterozygote; del, deletion.

tion. Enzymes from 13 of these patients exhibited altered electrophoretic and/or kinetic properties, suggesting a structural gene mutation. Recently, the molecular defect has been determined in a compound heterozygous case named "Hx Melzo" [74]. One allele has the 96-bp deletion within amino acids 162–193, and the other allele shows a single base substitution from T-to-C at position 1667, which causes the amino acid change from Leu to Ser at 529.

Glucosephosphate isomerase (GPI) deficiency is the fourth most common hereditary enzyme defect of red cells. So far, more than 40 unrelated families have been reported. It is inherited in an autosomal recessive manner, and about one-half of affected individuals are thought to be homozygous, while the other one-half appear to be compound heterozygotes. Although this enzyme is considered to be expressed virtually in all tissues, clinical manifestations are limited to hemolysis, with a few exceptions. Only two patients were mentally retarded, and one stored excess glycogen in an enlarged liver. A human cDNA for neuroleukin has proved to be identical with GPI. We have analyzed two homozygous variants: GPI Narita and GPI Matsumoto [75]. GPI Narita has a homozyous mutation from A to G at position 1028 (343 Gln to Arg). The substituted Gln is adjacent to the reported active site residue, 341 Asp. A homozygous missense mutation, C to T at position 14 (5 Thr to Ile), has been identified in GPI Matsumoto. This mutation seems to induce the enzyme instability, resulting in chronic hemolysis. In addition, two homozygous missense mutations and four compound heterozygous missense mutations have been determined [76,77].

Phosphofructokinase (PFK) deficiency is associated with a heterogenous group of clinical symptoms characterized by myopathy (Tarui disease) and/or hemolysis, or an asymptomatic state. More than 34 unrelated families with PFK deficiency have been described. According to the results of biochemical and immunological studies, clinical symptoms are considered to depend on the nature of defective isozymes. In two Japanese patients and an Ashkenazic Jewish family with typical clinical manifestations of Tarui disease, 5′-splice junction mutations resulting in splicing to a cryptic site within an exon, or exon skipping were identified [81–83]. These mutations lead to in-frame deletions, causing a severe deficiency of the PFK-M isozyme. Three other forms of mutations, splicing defects due to 3′-splice junction mutation, a nucleotide deletion resulting in a frameshift and premature termination, as well as missense mutations have been identified [78–81,84].

A deficiency of aldolase is a rare cause of hereditary hemolytic anemia. Only two families have been described. Nucleotide analysis of our patient revealed the substitution of a single nucleotide from A to G at position 386 within the coding region [85]. As a result, the 128th amino acid, Asp, was replaced by Gly. The mutated enzyme expressed in *E. coli* was thermolabile, as was the enzyme isolated from red cells of the patient. This demonstrated that a single base substitution was responsible for the pathogenesis of this disorder.

Triosephosphate isomerase (TPI) deficiency is an autosomal recessive disorder that has severe clinical manifestations, including hemolytic anemia, neurological dysfunction, sudden cardiac death, and increased suscep-

TABLE IV. Molecular Abnormalities of Other Enzyme Deficiencies

Mutant enzyme	Heredity	DNA defect	Deduced defect	Defect of other allele	Reference
Hx Melzo	Hetero	577–672 del	162–193 del	1667 T→C	74
Hx Melzo	Hetero	1667 T→C	529 Leu→Ser	577–672 del	74
GPI Matsumoto	Homo	14 C→T	5 Thr→Ile	14 C→T	75
GPI	Homo	247 C→T	83 Arg→Trp	247 C→T	76
GPI Narita	Homo	1028 A→G	343 Gln→Arg	1028 A→G	75
GPI	Homo	1574 T→C	524 Ile→Thr	1574 T→C	77
GPI	Hetero	475 G→A	158 Gly→Ser	1040 G→A	77
GPI	Hetero	671 C→T	224 Thr→Met	1483 G→A	76
GPI	Hetero	818 G→A	273 Arg→His	1039 C→T	76
GPI	Hetero	833 C→T	278 Ser→Leu	1459 C→T	76
GPI	Hetero	1039 C→T	347 Arg→Cys	818 G→A	76
GPI	Hetero	1040 G→A	346 Arg→His	475 G→A	77
GPI	Hetero	1459 C→T	487 Leu→Phe	833 C→T	76
GPI	Hetero	1483 G→A	495 Glu→Lys	671 C→T	76
PFK	Homo	116 G→C	39 Arg→Pro	116 G→C	78
PFK	Homo	116 G→T	39 Arg→Leu	116 G→T	79
PFK	Homo	626 G→A	209 Gly→Asp	626 G→A	80
PFK	Homo	2003 C del	fs-pt	2003 C del	79
PFK	Homo	IVS-5 (5′ end) G→A	Exon 5 del	IVS-5 (5′ end) G→A	81
PFK	Homo	IVS-6 (3′ end) A→C	Two deleted transcripts	IVS-6 (3′ end) A→C	78
PFK	Homo	IVS-15 (5′ end) G→T	25 amino acids del	IVS-15 (5′ end) G→T	82
PFK	Homo	IVS-19 (5′ end) G→A	Exon 19 del	IVS-19 (5′ end) G→A	83
PFK	Hetero	299 G→A	100 Arg→Gln	2087 G→A	80
PFK	?	1127 G→A	376 Arg→Gln	?	84
PFK	Hetero	1628 A→C	543 Asp→Ala	mRNA(−)	78
PFK	?	2058 G→T	686 Trp→Cys	?	84
PFK	Hetero	2087 G→A	696 Arg→His	299 G→A	80
ALD	Homo	386 A→G	128 Asp→Gly	386 A→G	85
TPI	Homo	314 G→C	104 Glu→Asp	314 G→C	86,87
TPI Manchester	Homo	366 G→A	122 Gly→Arg	366 G→A	88
TPI	Homo	693 G→A	231 Val→Met	693 G→A	89
TPI	Hetero	567 C→T	Nonsense	314 G→C	90,91
TPI	Hetero	720 T→C	240 Phe→Leu	Reduced mRNA	92
DPGM	Hetero	205 or 206 C del	fs-pt	413 C→T	93
DPGM	Hetero	413 C→T	89 Arg→Cys	205 or 206 C del	93
PGK Matsue	Hemi	266 T→G	88 Leu→Pro	—	94
PGK North Carolina	Hemi	IVS-4 (5′ end) G→T	10 amino acids ins	—	95
PGK Shizuoka	Hemi	473 G→T	157 Gly→Val	—	96
PGK Amiens	Hemi	490 A→T	163 Asp→Val	—	97
PGK Uppsala	Hemi		205 Arg→Pro	—	98
PGK Tokyo	Hemi		265 Val→Met	—	99
PGK München	Hemi		267 Asp→Asn	—	100
PGK Créteil	Hemi	942 G→A	314 Asp→Asn	—	97
PGK Michigan	Hemi	946 A→G	315 Cys→Arg	—	101
PGK II	Hemi		351 Thr→Asn	—	102
AK	Hetero	382 C→T	128 Arg→Trp	Normal	103

Hx, hexokinase; GPI, glucosephosphate isomerase; PFK, phosphofructokinase; ALD, aldolase; TPI, triosephosphate isomerase; DPGM, diphosphoglyceromutase; PGK, phosphoglycerate kinase; AK, adenylate kinase; Homo, homozygote; Hetero, heterozygote; Hemi, hemizygote; del, deletion; fs-pt, frameshift→premature termination; ins, insertion.

tibility to infection. More than 25 unrelated families have been described. A TPI deficiency has been attributed to either homozygosity for missense mutations within codons 104, 122, or 231, or compound heterozygosity for missense mutation (codon 104)/nonsense mutation or missense mutation (codon 240)/reduced mRNA have been clarified [86–92].

Red cell diphosphoglyceromutase (DPGM) deficiency is a rare disease associated with a decreased 2,3-diphosphoglycerate concentration. A complete deficiency of DPGM was discovered in a man of French origin associated with a moderate erythrocytosis. Sequence studies of this individual indicated heterozygosity with a cytidine to thymine substitution at nucleotide 413 and another heterozygosity with the deletion of cytidine at nucleotide 205 or 206 [93]. Therefore, the complete enzyme deficiency resulted from a genetic compound with one allele coding for a missense mutation (Arg to Cys at 89) and

the other bearing a frameshift and premature termination of translation.

A hereditary phosphoglycerate kinase (PGK) deficiency is associated with hemolytic anemia, neurological manifestations and often myopathy with recurrent myoglobinuria. This deficiency is inherited as an X-linked disorder, and 18 variants are known. Six of these patients have manifested both symptoms, six have shown only hemolysis, five have shown neuromuscular dysfunction but without hemolysis, and one was without clinical symptoms. Missense mutations have been identified in PGK Matsue, PGK Shizuoka, PGK Amiens, PGK Uppsala, PGK Tokyo, PGK München, PGK Créteil, PGK Michigan, and PGK II [94,96–102]. A G-to-T substitution at the 5′ end of intron 4 has been identified in PGK North Carolina [95]. This mutation destroys the consensus sequence GT at the 5′ splice junction of the intron. Activation of a cryptic splice site within intron 4 causes the insertion of a 30-bp fragment at the 5′ end of intron 4 into the transcript. These studies have clarified the correlation between the functional and structural abnormalities of the variant enzymes. In general, the substitutions that induced a dislocation of the nucleotide or substrate binding site of the enzyme resulted in markedly altered enzyme properties and severe clinical symptoms.

Red cell adenylate kinase (AK) deficiency is a rare genetic disorder. So far only four examples have been reported, three of which were associated with hemolysis, the fourth showing no evidence of hemolysis despite undetectable red cell AK activity. Nucleotide analysis of our patient revealed a single nucleotide substitution from C to T, resulting in a change of Arg to Trp at the 128th amino acid residue [103].

OTHER ENZYME DEFICIENCIES WITH UNDEFINED MOLECULAR DEFECTS

Pyrimidine 5′-nucleotidase (P5N) deficiency appears to be the third most common cause of erythroenzymopathies associated with hemolysis. This syndrome is characterized by hemolytic anemia, pronounced basophilic stippling of red cells, and marked increases in red cell reduced glutathione and in pyrimidine-containing nucleotides. To date, more than 42 individuals have been described worldwide. The main cause of a P5N deficiency is considered to be an abnormality of P5N-I [104], probably arising from a structural gene mutation. The precise molecular defect has not been clarified, because a normal gene for P5N-I has not been isolated.

Markedly increased adenosine deaminase (ADA) activity and decreased ATP in red cells develops into hereditary hemolytic anemia. The mode of inheritance is autosomal dominant. Only four families have been described, including our two. ADA activity increases on the order of a hundredfold, whereas the concentration of ATP in the patients' red cells was about half that of comparably reticulocyte-rich control. In general, anemia was mild or fully compensated with reticulocytosis, hyperbilirubinemia, and a shortened red cell half-life. Red cell morphology in our patients revealed stomatocytosis. The defect may be tissue specific, because ADA activity in the leukocytes and skin fibroblasts was normal. The basic abnormality appears to result from overproduction of a structurally normal enzyme. Recently, increased TAAA repeats located at the tail end of an *Alu* repeat about 1.1 kb upstream of the ADA gene have been identified in affected individuals [105]. This *cis*-acting mutation might cause the overexpression of ADA in red blood cells.

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